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Differences in mucin levels and bacterial patterns in the sputum of smokers and Non-Smokers: A Cross-Sectional study

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Abstract

Cigarette smoke exposure is known to induce airway inflammation and mucus hypersecretion and may alter respiratory bacterial colonization. However, data regarding early mucin alterations and airway bacterial patterns among otherwise healthy individuals remain limited, particularly in settings with high smoking prevalence. This study aimed to analyze differences in mucin levels and bacterial patterns in induced sputum samples between smokers and non-smokers among healthy young adults in Surabaya, Indonesia. An analytical observational study with a cross-sectional design was conducted among 68 adult participants, consisting of 34 smokers and 34 non-smokers. Sputum samples were analyzed for mucin levels and bacterial patterns. Data were analyzed using SPSS. Normality was assessed using the Shapiro–Wilk test. Comparisons between groups were performed using the Mann–Whitney U test and chi-square test. Statistical significance was set at $p<0.05$. The mean mucin level among smokers was 1.37 ± 0.45 compared to 1.47 ± 0.53 in non-smokers ($p = 0.545$). The prevalence of pathogenic bacteria was 60.3% overall, with no significant difference between smokers and non-smokers ($p = 0.806$). Normal flora was present in 97.1% of participants, predominantly Streptococcus viridans. No significant correlation was found between mucin levels and bacterial presence ($r = –0.102$, $p = 0.406$). There were no statistically significant differences in mucin levels or bacterial patterns between smokers and non-smokers in this study. These findings suggest that smoking exposure in this relatively young population may not yet have resulted in measurable alterations in sputum mucin levels or bacterial colonization.

Keywords: Smoking, Mucin, Sputum, Bacterial colonization, Airway microbiota

Introduction

Cigarette smoking remains one of the leading preventable causes of morbidity and mortality worldwide, contributing significantly to chronic respiratory diseases, cardiovascular disorders, and malignancy.[1] According to the World Health Organization, the global burden of tobacco use continues to affect millions of individuals, with Southeast Asia demonstrating persistently high smoking prevalence rates.[2] Indonesia, in particular, has one of the highest smoking rates globally, especially among adult males.[3]

Chronic exposure to cigarette smoke induces airway epithelial injury, oxidative stress, and persistent inflammation. These processes stimulate goblet cell hyperplasia and mucus hypersecretion, leading to increased mucin production and impaired mucociliary clearance.[4,5] Alterations in mucin expression, particularly MUC5AC and MUC5B, have been implicated in airway remodeling and the pathogenesis of chronic bronchitis and chronic obstructive pulmonary disease (COPD).[6,7]

Mucins are high-molecular-weight glycoproteins that form the structural backbone of airway mucus and play a crucial role in host defense.[8] However, dysregulated mucin expression may compromise mucociliary function and promote bacterial colonization.[9] Experimental and clinical studies have demonstrated that cigarette smoke exposure may modify mucin gene expression and alter airway secretory function even before overt lung function impairment becomes evident.[10]

In addition to mucus alterations, smoking has been associated with changes in airway microbial ecology. Cigarette smoke may impair innate immune responses, disrupt epithelial barrier integrity, and alter the composition of airway microbiota.[11,12] While most studies focus on patients with established chronic respiratory diseases, limited data are available regarding mucin levels and bacterial patterns among relatively healthy smokers without clinically apparent pulmonary disease.

Furthermore, environmental factors such as secondhand smoke exposure and urban air pollution may independently influence airway inflammation

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and mucus production.[13,14] In urban settings such as Surabaya, background exposure to environmental pollutants may contribute to airway irritation even among non-smokers.

Given the high prevalence of smoking in Indonesia and the potential interaction between smoking, environmental exposure, mucin production, and airway microbiota, understanding early airway changes among healthy individuals is essential. Therefore, this study aimed to evaluate differences in mucin levels and bacterial patterns in induced sputum samples between smokers and non-smokers in a relatively healthy young adult population.

Methods

Study design and participants

This analytical observational study employed a cross-sectional design. A total of 68 participants were included, consisting of 34 smokers and 34 non-smokers. The mean age of smokers was 31.65 ± 6.19 years, and non-smokers 31.76 ± 5.31 years. All participants were male. Smokers had a mean smoking duration of 14.32 ± 5.86 years and an average cigarette consumption of 17.82 ± 1.99 sticks per day. Participants with a history of chronic pulmonary disease, abnormal chest radiographs, positive COVID-19 PCR results, or abnormal spirometry were excluded.

Sputum collection

Sputum samples were obtained through sputum induction using nebulized hypertonic saline under medical supervision. Prior to the procedure, participants were instructed to rinse their mouths with clean water to minimize oral contamination. Induced sputum was collected in sterile containers during morning hours. Samples with adequate volume and visible lower respiratory tract secretions were considered acceptable for analysis. All specimens were transported promptly to the laboratory for mucin evaluation and bacterial culture.

Mucin analysis

Induced sputum samples were processed for mucin evaluation. Smears were prepared and stained using Periodic Acid–Schiff (PAS) staining to visualize mucin content. The slides were examined under light microscopy at ×400 magnification.

Mucin levels were categorized into three categories based on staining intensity and distribution pattern. Category A was defined as sparse and lightly stained mucin with minimal aggregation. Category B was defined as moderate mucin accumulation with visible filamentous structures. Category C was defined as dense, intensely stained, and homogeneous mucin deposition.

All slides were evaluated by a trained examiner who was blinded to participants' smoking status to minimize observer bias.

Bacterial culture

Induced sputum samples were cultured using standard microbiological techniques. Specimens were inoculated onto blood agar and MacConkey agar plates and incubated at 37°C under aerobic conditions for 24–48 hours. After incubation, bacterial growth was assessed based on colony morphology, Gram staining, and conventional biochemical identification methods.

The presence of pathogenic bacteria and normal flora was recorded for each participant. Identified bacterial species were documented for descriptive analysis.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics version 29 (IBM Corp., Armonk, NY, USA). Continuous variables were assessed for normality using the Shapiro–Wilk test. As most numerical variables were not normally distributed, comparisons between smokers and non-smokers were conducted using the Mann–Whitney U test. Categorical variables were analyzed using the Chi-square test or Fisher's exact test as appropriate.

Continuous data are presented as mean ± standard deviation or median (interquartile range), while categorical variables are presented as frequency and percentage. All statistical tests were two-tailed, and a p-value < 0.05 was considered statistically significant.

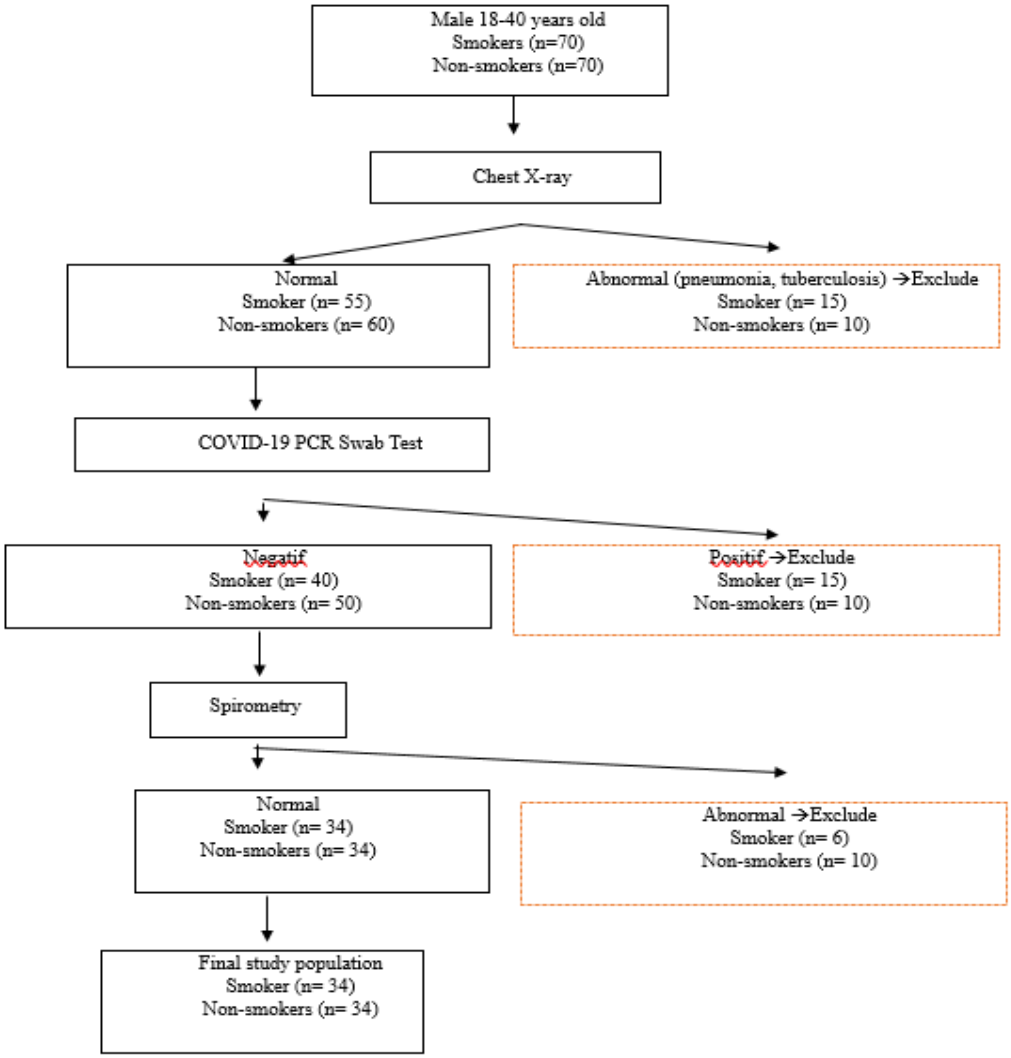

Fig 1

Results

Baseline characteristics

A total of 68 participants were included, consisting of 34 smokers and 34 non-smokers. No significant age difference was observed between smokers and non-smokers (p = 0.883). Body mass index was significantly higher in non-smokers (26.94 ± 6.59 kg/m^2) compared to smokers (23.11 ± 4.15 kg/m^2; p = 0.005).

Table 1. Baseline characteristics of study participants

Variable	Smokers (n = 34)	Non-smokers (n = 34)	p-value
Age (years), mean ± SD	31.65 ± 6.19	31.76 ± 5.31	0.883†
Body Mass Index (kg/m^2), mean ± SD	23.11 ± 4.15	26.94 ± 6.59	0.005*†
Smoking duration (years), mean ± SD	14.32 ± 5.86	—	—
Cigarettes per day, mean ± SD	17.82 ± 1.99	—	—
Sex (male), n (%)	34 (100)	34 (100)	—

Values are presented as mean ± standard deviation unless otherwise indicated. † Mann–Whitney U test
* Statistically significant at p < 0.05.

Mucin levels

The mean mucin level among smokers was 1.37 ± 0.45, while non-smokers had 1.47 ± 0.53. Mann–Whitney analysis showed no significant difference (U=529.0, p = 0.545). Similarly, mucin category distribution did not differ significantly between groups (p = 0.563).

Table 2. Comparison of sputum mucin levels between smokers and Non-Smokers

Variable	Smokers (n = 34)	Non-smokers (n = 34)	p-value
Mucin level, mean ± SD	1.37 ± 0.45	1.47 ± 0.53	0.545†
Mucin level, median (IQR)	1.15 (1.00–1.80)	1.30 (1.00–1.90)	—
Mucin category, n(%)			0.563†
– Category A	25 (73.5)	23 (67.6)	
– Category B	8 (23.5)	9 (26.5)	
– Category C	1 (2.9)	2 (5.9)	

Values are presented as mean ± standard deviation or median (interquartile range).
† Mann–Whitney U test

Representative photomicrographs demonstrating the grading criteria of sputum mucin are presented in Figure 1. Category A shows sparse and lightly stained mucin with minimal aggregation. Category B demonstrates moderate mucin accumulation with visible filamentous structures. Category C is characterized by dense, intensely stained, and homogeneous mucin deposition.

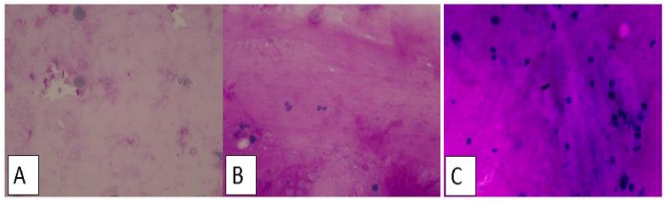

Figure 1. Representative microscopic images of sputum mucin categories

The representative photomicrographs further illustrate the progressive increase in mucin density across categories, supporting the categorization criteria applied in this study. Although no statistically significant difference in mucin levels was observed between smokers and non-smokers, the morphological spectrum demonstrated in Figure 1 confirms the consistency of the mucin classification used in sputum analysis.

Bacterial patterns: Pathogenic bacteria were detected in 20 (58.8%) smokers and 21 (61.8%) non-smokers, with no statistically significant difference between groups (p = 0.806). The most frequently isolated pathogen in both groups was Klebsiella pneumoniae. Normal flora was identified in 97.1% of samples in both groups, and no significant association was observed between smoking status and normal flora presence (p = 1.000). The detailed distribution of pathogenic bacterial species and normal flora is presented in Table 3.

Table 3. Distribution of bacterial patterns in smokers and Non-smokers

Variable	Smokers (n = 34)	Non-smokers (n = 34)	p-value
Pathogenic bacteria present, n (%)	21 (61.8)	20 (58.8)	0.806†
Pathogenic species identified, n (%):			
Klebsiella pneumoniae	10 (29.4)	11 (32.4)	—
Pseudomonas aeruginosa	2 (5.9)	1 (2.9)	—
Enterobacter cloacae	2 (5.9)	—	—
Staphylococcus aureus	1 (2.9)	—	—
Staphylococcus spp.	2 (5.9)	1 (2.9)	—
Streptococcus pyogenes	1 (2.9)	1 (2.9)	—
Acinetobacter baumannii	1 (2.9)	—	—
Acinetobacter junii	1 (2.9)	—	—
Escherichia coli	—	1 (2.9)	—
Klebsiella aerogenes	—	1 (2.9)	—
Klebsiella sp.	—	1 (2.9)	—
Pseudomonas alcaligenes	—	1 (2.9)	—
Stenotrophomonas maltophilia	—	2 (5.9)	—
Streptococcus dysgalactiae	—	1 (2.9)	—
Normal flora present, n (%)	33 (97.1)	33 (97.1)	1.000†
Streptococcus viridans, n (%)	32 (94.1)	33 (97.1)	0.554†

Values are presented as frequency (percentage).
† Chi-square test or Fisher's exact test as appropriate

Correlation analysis

No significant correlation was found between mucin levels and pathogenic bacteria ($r = -0.102$, $p = 0.406$). This suggests that mucin categorization was not associated with the presence of pathogenic bacterial colonization in this population.

Discussion

This study evaluated differences in sputum mucin levels and bacterial patterns between smokers and non-smokers among healthy young adults in Surabaya, Indonesia. The findings demonstrated no statistically significant difference in mucin levels or distribution of pathogenic bacteria between the two groups. However, Body Mass Index (BMI) was significantly higher among non-smokers.

The absence of a significant difference in mucin levels may be explained by the relatively young age and healthy status of the participants. With a mean age of approximately 31 years, cumulative exposure to cigarette smoke may not yet have resulted in measurable structural or secretory changes in airway mucin production. Chronic smoking has been associated with goblet cell hyperplasia and mucin overproduction, particularly in individuals with established airway disease such as chronic bronchitis and chronic obstructive pulmonary disease (COPD).[1,2] In contrast, participants in this study were rigorously screened to exclude underlying pulmonary pathology, including abnormal chest radiographs and spirometric impairment, which may explain the lack of observable mucin alteration.

Furthermore, strict inclusion criteria were applied to ensure sample homogeneity. Individuals with a history of pulmonary disease, abnormal chest radiographic findings, positive COVID-19 PCR results, or abnormal spirometry were excluded. This approach minimized confounding respiratory conditions that could independently influence mucin production.

Importantly, this study was conducted in Surabaya, Indonesia, a setting characterized by a high national smoking prevalence.[3] Although the non-smoker group did not actively smoke, passive exposure to secondhand smoke cannot be entirely excluded, particularly in communities with substantial environmental tobacco exposure. Secondhand smoke has been shown to induce airway inflammation and mucus secretion,[4,15] which may attenuate differences between active smokers and non-smokers.

In addition, urban air pollution in Surabaya may contribute to airway irritation and mucus production in both groups. Exposure to particulate matter and traffic-related air pollutants has been associated with airway inflammation and increased mucin expression.[5,6,16] Background environmental exposure may therefore partially mask smoking-specific effects in a relatively healthy young population.

Similarly, no significant difference was observed in the overall presence of pathogenic bacteria between smokers and non-smokers. Although smoking has been linked to impaired mucociliary clearance and alterations in airway microbiota,[7,17,18] such changes are more pronounced in individuals with chronic respiratory disease. The predominance of normal flora and the comparable distribution of pathogenic species, particularly *Klebsiella pneumoniae*, suggest that smoking exposure in otherwise healthy adults may not yet substantially alter colonization patterns.

Community antibiotic practices may also influence bacterial findings. In Indonesia, non-prescription antibiotic use and premature discontinuation of therapy remain public health concerns.[8,19,20] Such patterns may contribute to altered bacterial colonization even among healthy individuals, potentially explaining the presence of pathogenic bacteria in both groups.

The significantly higher BMI observed among non-smokers aligns with previous evidence suggesting that smoking influences appetite regulation and metabolic pathways.[9,21,22] Although BMI was not a primary outcome of this study, this finding highlights the complex interaction between smoking behavior and metabolic status.

Several limitations should be considered. The cross-sectional design precludes causal inference between smoking exposure and mucin production or bacterial colonization. The relatively small sample size may limit statistical power to detect subtle differences. Additionally, the study population consisted primarily of young adults, some of whom were hospital employees, which may limit generalizability

to older individuals or those with long-term heavy smoking exposure.

Despite these limitations, this study provides insight into early airway mucin characteristics and bacterial patterns among healthy young adults in an urban Indonesian setting. Future studies involving larger cohorts and individuals with longer smoking histories may better elucidate early mucin alterations and microbiological changes associated with smoking.

Strengths and limitations

This study has several strengths. Strict inclusion criteria were applied to ensure a homogeneous population of healthy young adults, minimizing confounding respiratory conditions. The use of induced sputum and standardized laboratory procedures for mucin categorization and bacterial culture enhanced the reliability of the findings.

However, several limitations should be acknowledged. The cross-sectional design precludes causal inference between smoking exposure and airway mucin production. The relatively small sample size may limit statistical power to detect subtle differences. In addition, passive smoke exposure and environmental air pollution were not quantitatively measured, which may have influenced the findings. The study population, which included hospital employees, may also limit generalizability to broader community settings.

Conclusion

In this study of healthy young adults in Surabaya, Indonesia, no significant differences were observed in sputum mucin levels or bacterial patterns between smokers and non-smokers. These findings suggest that in relatively young individuals without underlying pulmonary disease, smoking exposure may not yet produce measurable alterations in mucin categorization or airway bacterial colonization. Further studies involving larger populations and individuals with longer smoking histories are needed to better understand early airway changes associated with smoking.

These findings highlight the importance of considering environmental and demographic factors when evaluating early airway changes associated with smoking.

Ethical approval

Approved by the Health Research Ethics Committee of Dr. Soetomo General Hospital, Surabaya (Approval No. 0843/KEPK/XI/2023).

Statement of authorship

All authors certified fulfillment of ICMJE authorship criteria.

Author disclosure

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